CNRS
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Université Louis Pasteur

Parc d'Innovation - 1, rue Laurent Fries - ILLKIRCH - CU. DE STRASBOURG - FRAN

Traitement d'image
et reconstruction tri-dimensionnelle

ReNaFoBis Ile d'Oléron 5th June 2014

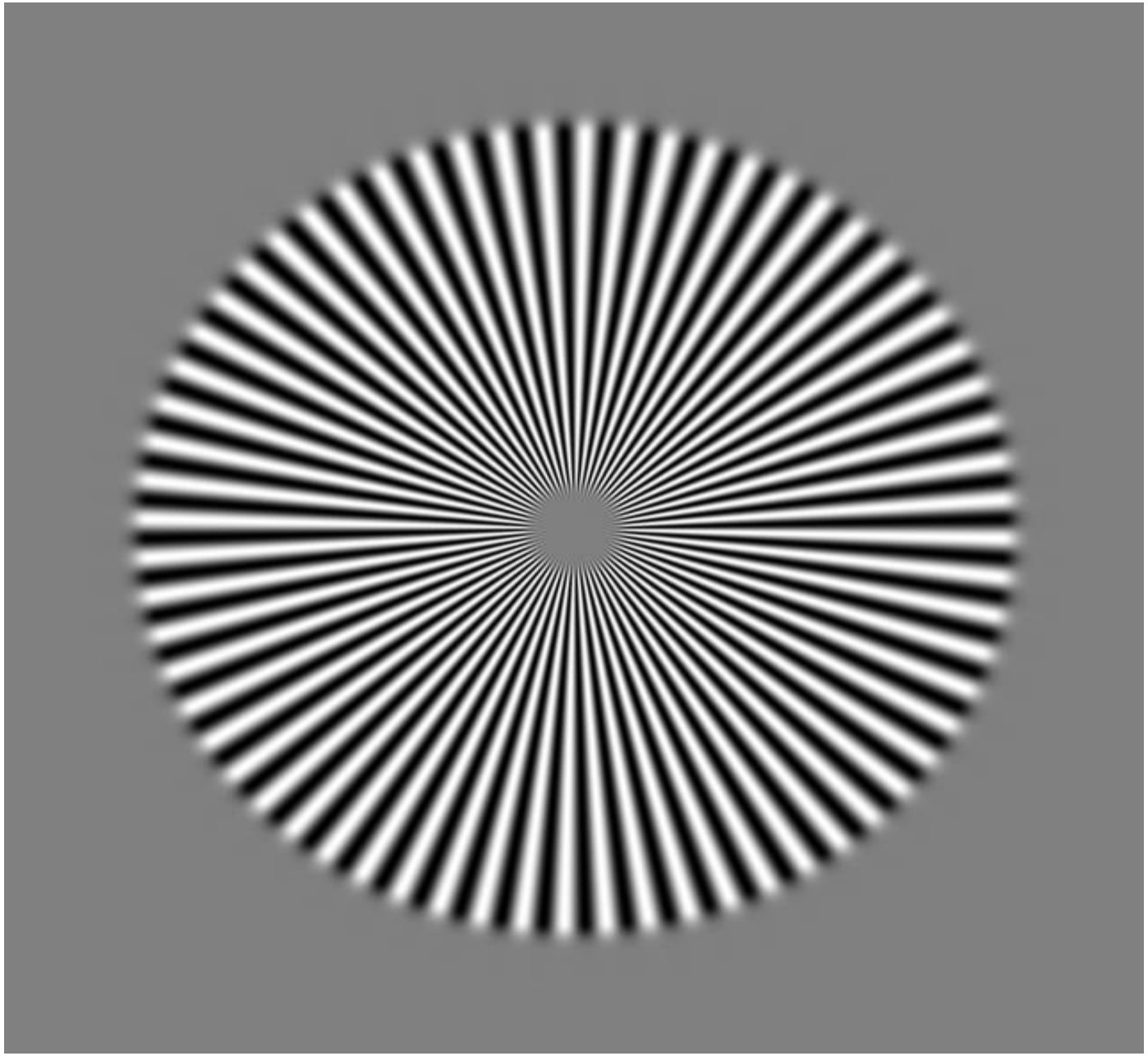
Jean-François Ménétret

for Bruno Klaholz

http://www.igbmc.fr

http://igbmc.fr/Klaholz

Siemens stars: a whole range of spacings / frequencies in a single image



One wedge every 1.5 degree

It is used to test the resolution of optical instruments.

drawn by M. van Heel

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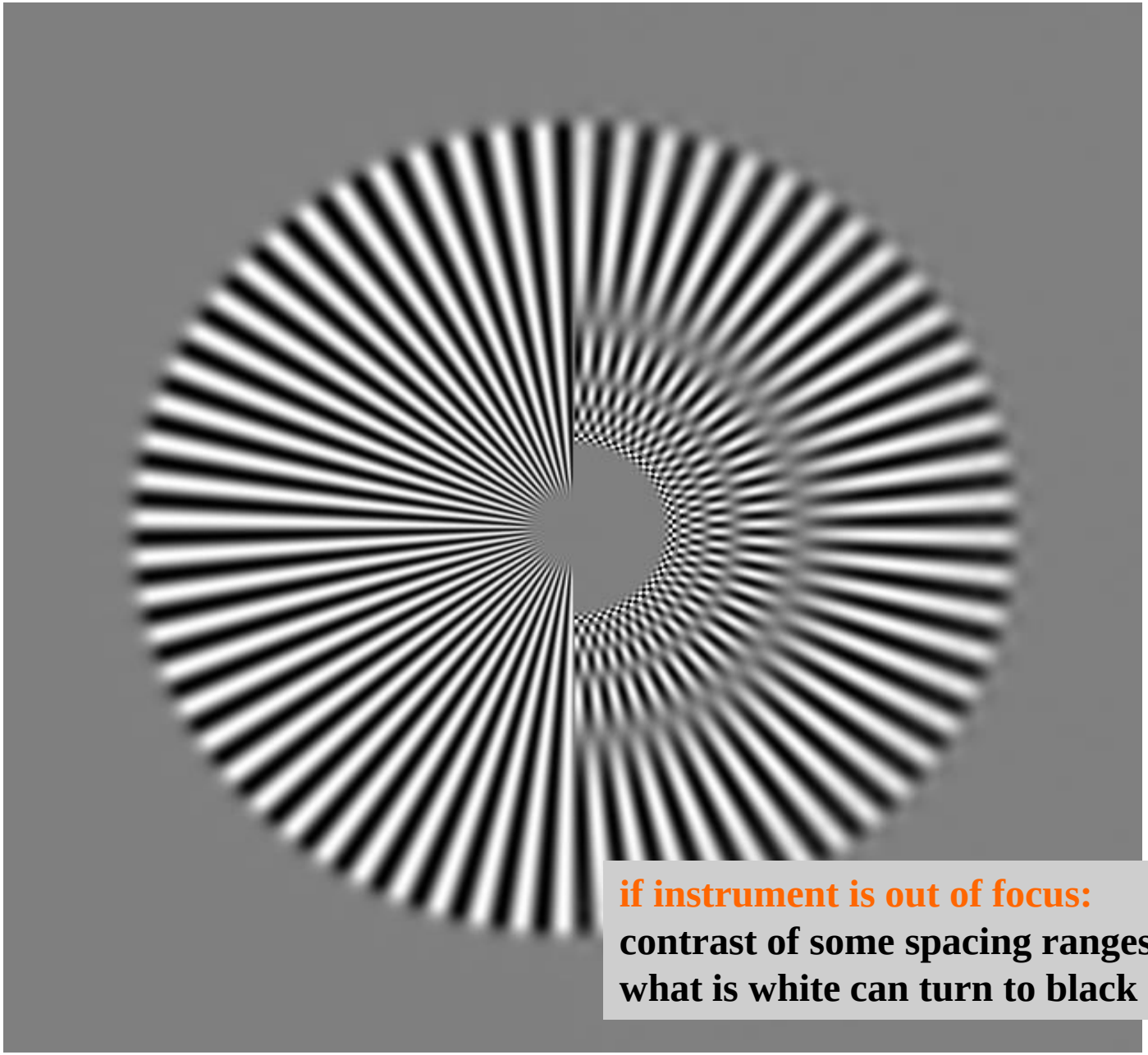
Calculated effect of an electron microscopical PhCTF on the image of a Siemens star



if instrument is out of focus:

contrast of some spacing ranges may be reversed
what is white can turn to black and vice versa.

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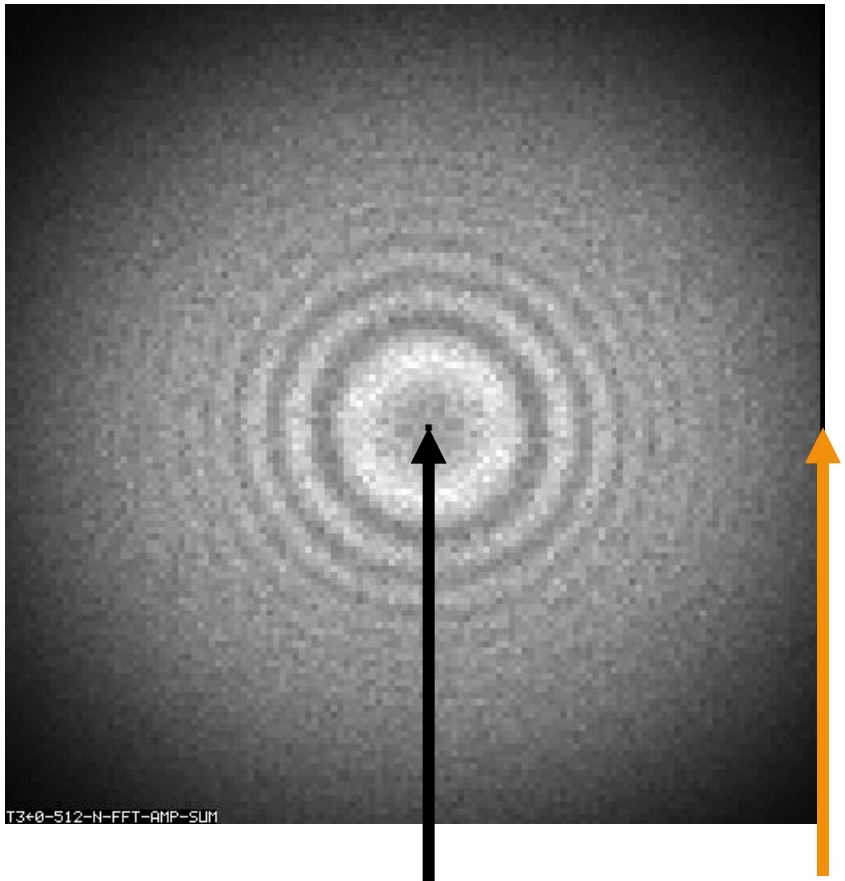
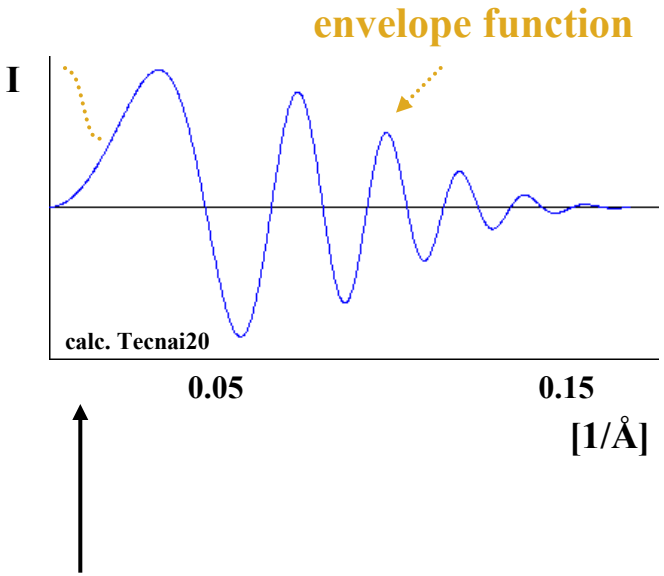
Powerspectrum = Fourier Transformation of the image



low resolution
low frequency

high resolution
high frequency

Profile of the intensity distribution



very low resolution difficult to measure

→ averaging techniques

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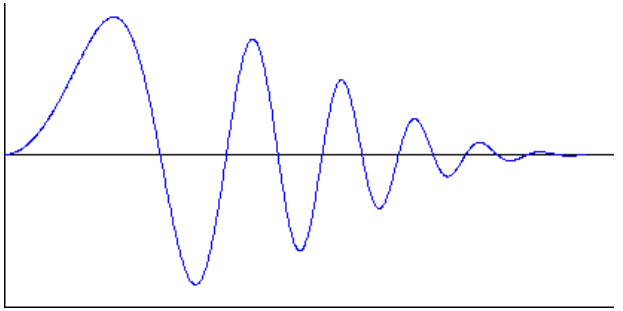
I. Pre-processing

- **correction of the Contrast Transfer Function**



Weak-phase biological specimen → weak-contrast image

$$\mathrm{CTF}(f) = A(\sin(\pi\lambda f^2(\Delta z - 0.5\lambda^2 f^2 c_s)) + B\cos(\pi\lambda f^2(\Delta z - 0.5\lambda^2 f^2 c_s)))$$

Phase contrast and amplitude contrast

λ wavelength defined by accelerating voltage (e.g. high tension 200 kV --> λ = 0.025 Å)

***Cs* spherical aberration coefficient, determines the quality of objective lens**

***B* fraction of amplitude contrast**

***A* defocus-dependent envelope function**

Δz: the defocus value, (offset from focus, "Scherzer" focus, underfocus: focal point is below the sample)

***f*, spatial frequency**

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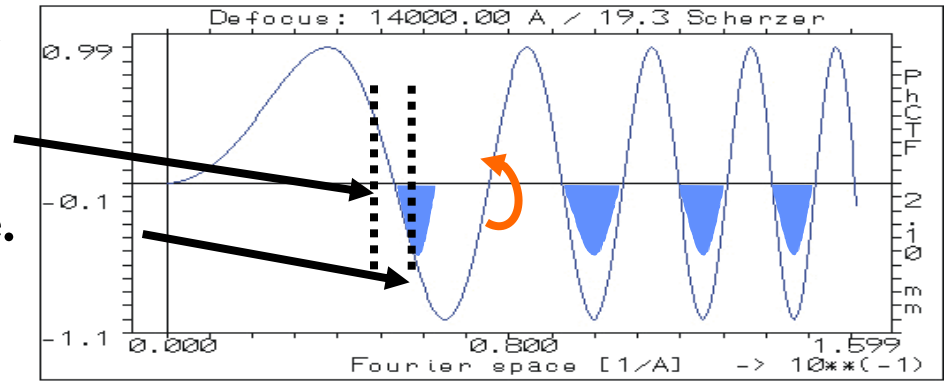
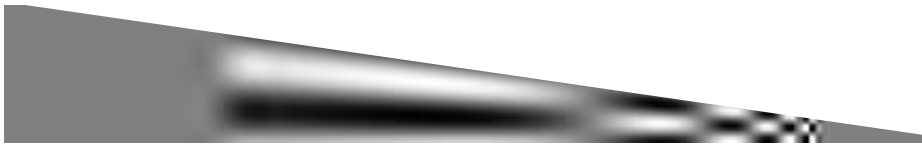
I. Pre-processing

- correction of the contrast transfer function (CTF)

No information transfer at the zero crossing;
CTF leads to contrast inversion within the image.

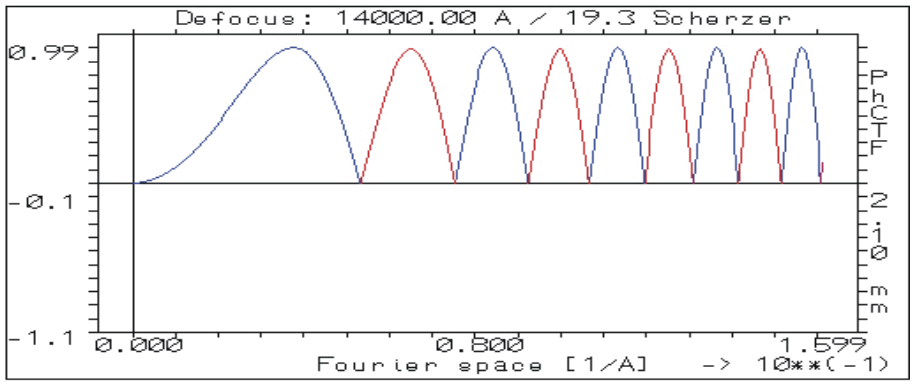
A





Radial segment of Siemens star

B



CTF-correction? By "phase flipping":

Therefore: data collection over range of defocus values,
e.g. under-focus -1.0 – 3.0 µm

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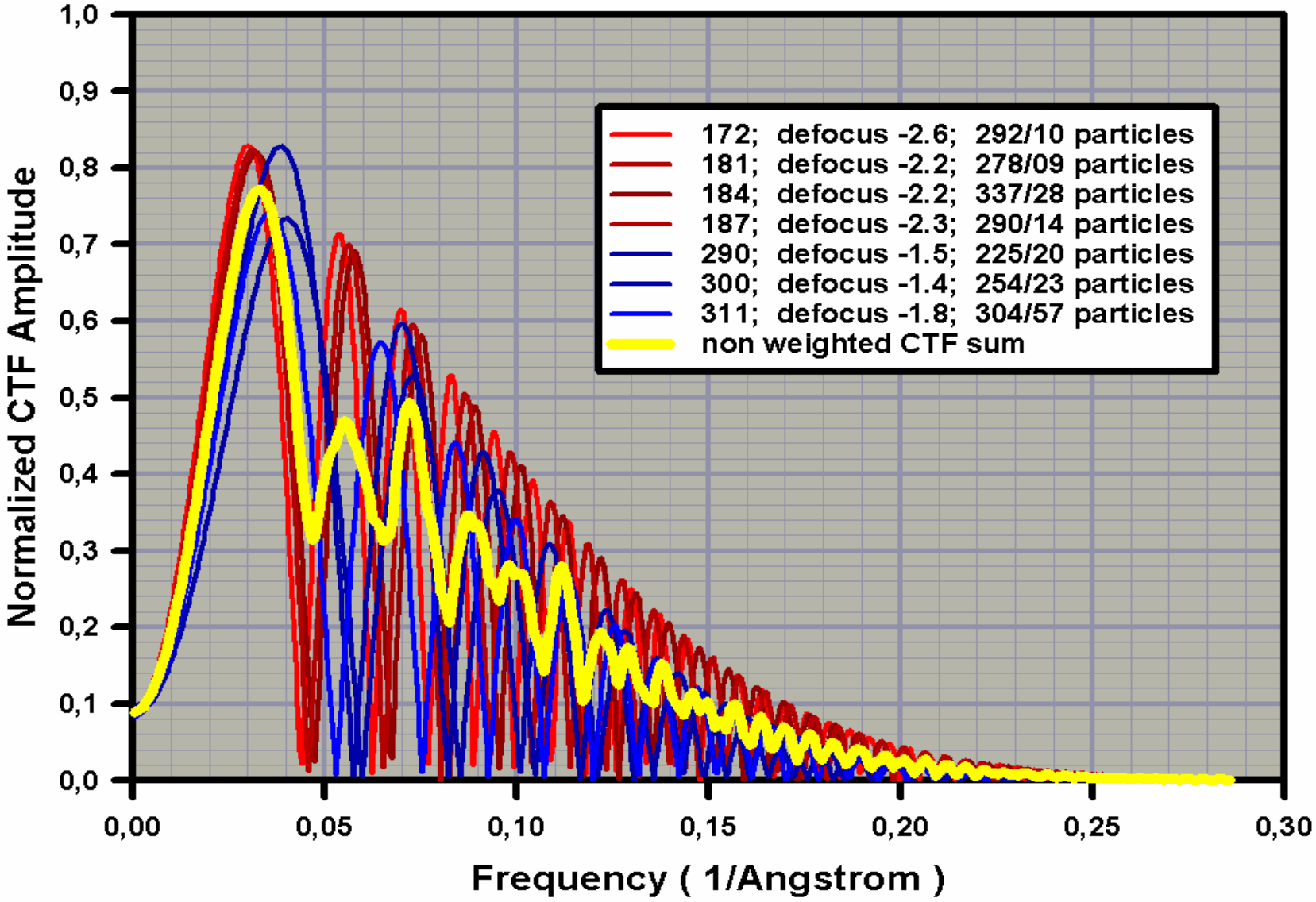
Combination of powerspectra from different defocus images



(spectra also take the envelope function into account)

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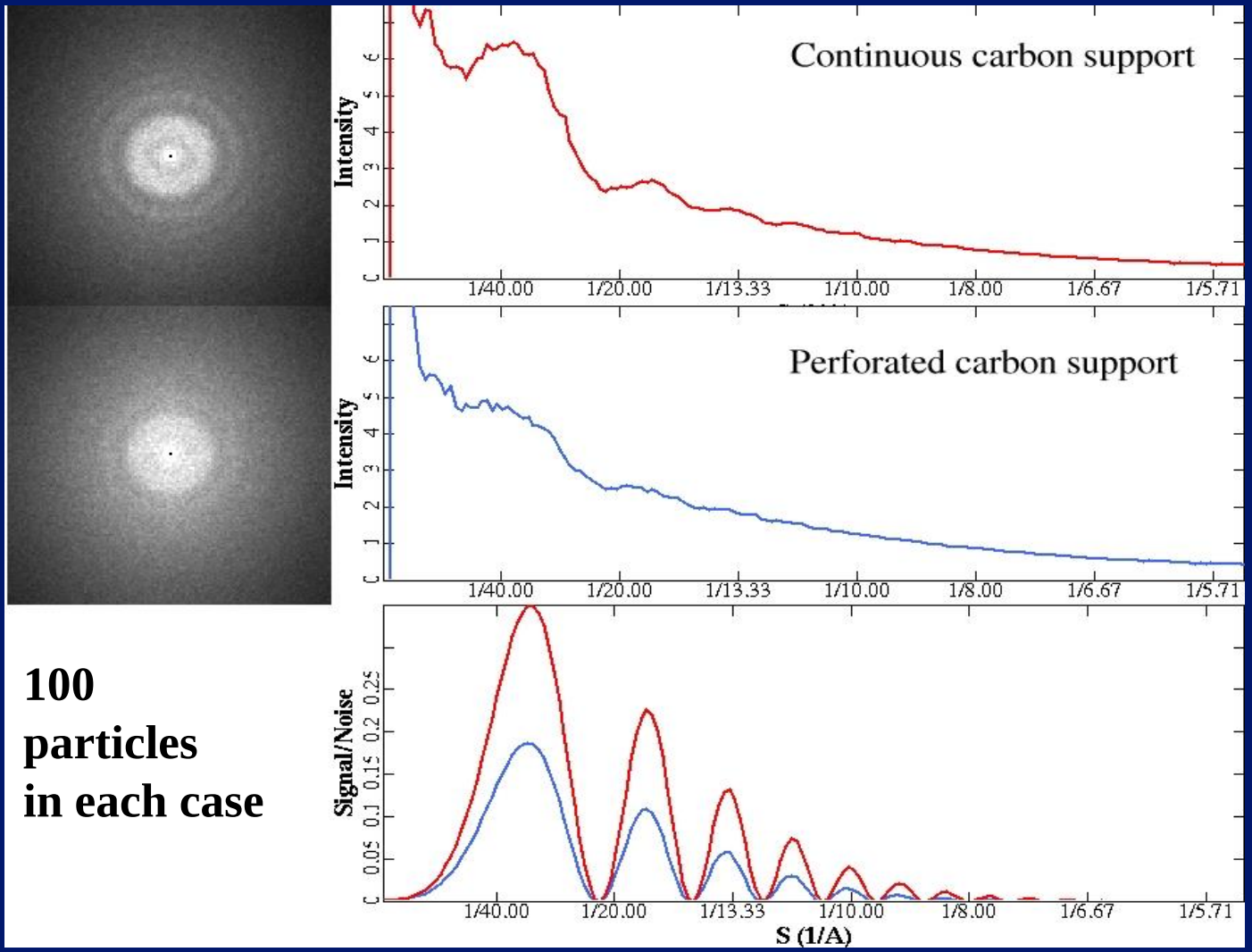
In EMAN2 on Virtual Machine

1) **display images**
2) **calculate their FFT**
3) **to see the CTF + noise + structure factor**

In EMAN1 on Virtual Machine

1) **simulate the CTF, envelope function, Nyquist limit**
2) **add several CTF curves**
3) **See complementarity between curves at different defocus values Δf**

Cryo-EM; Contrast Transfer Function



100 particles in each case

Curves include:

CTF,

background noise,

structure factor,

envelope function

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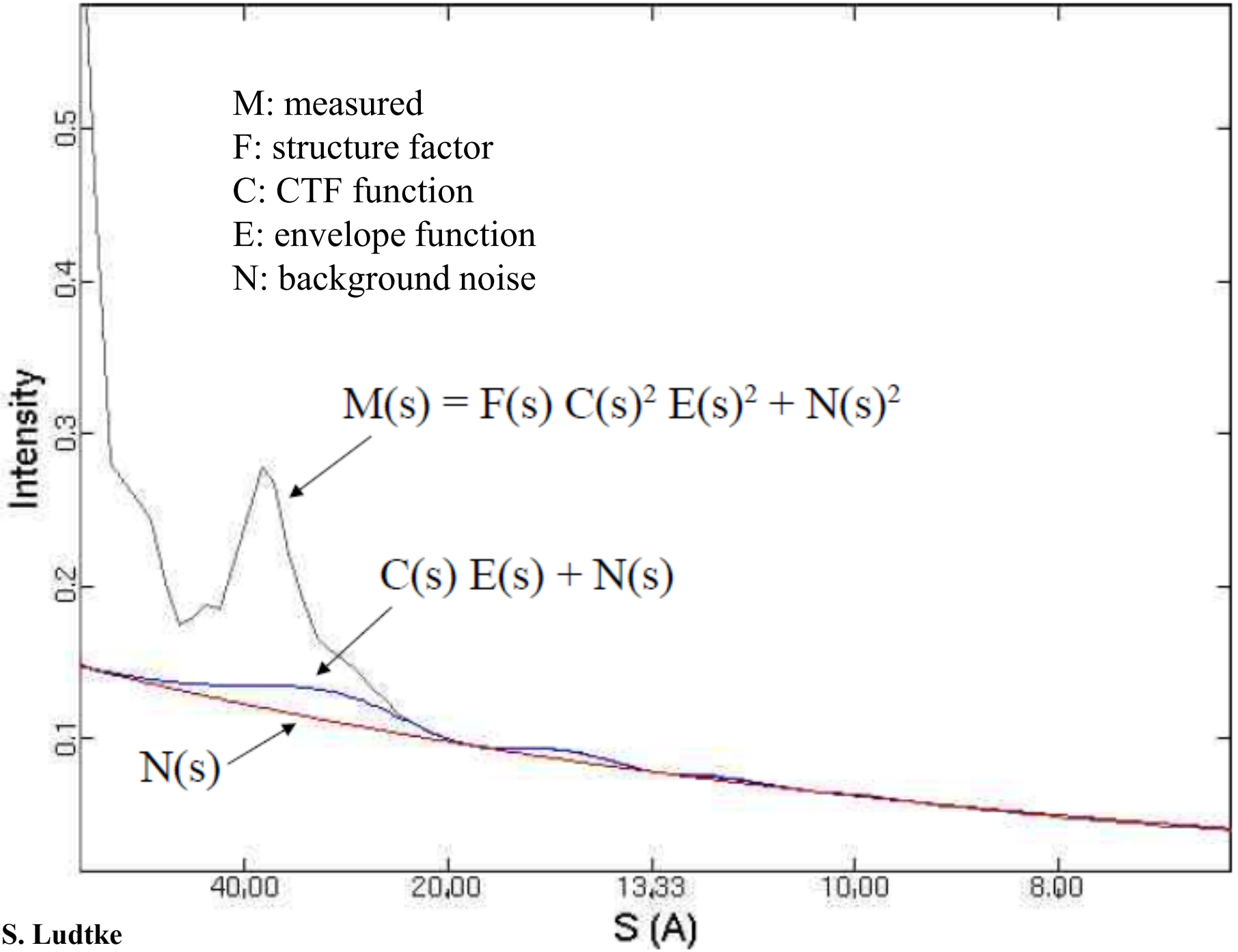
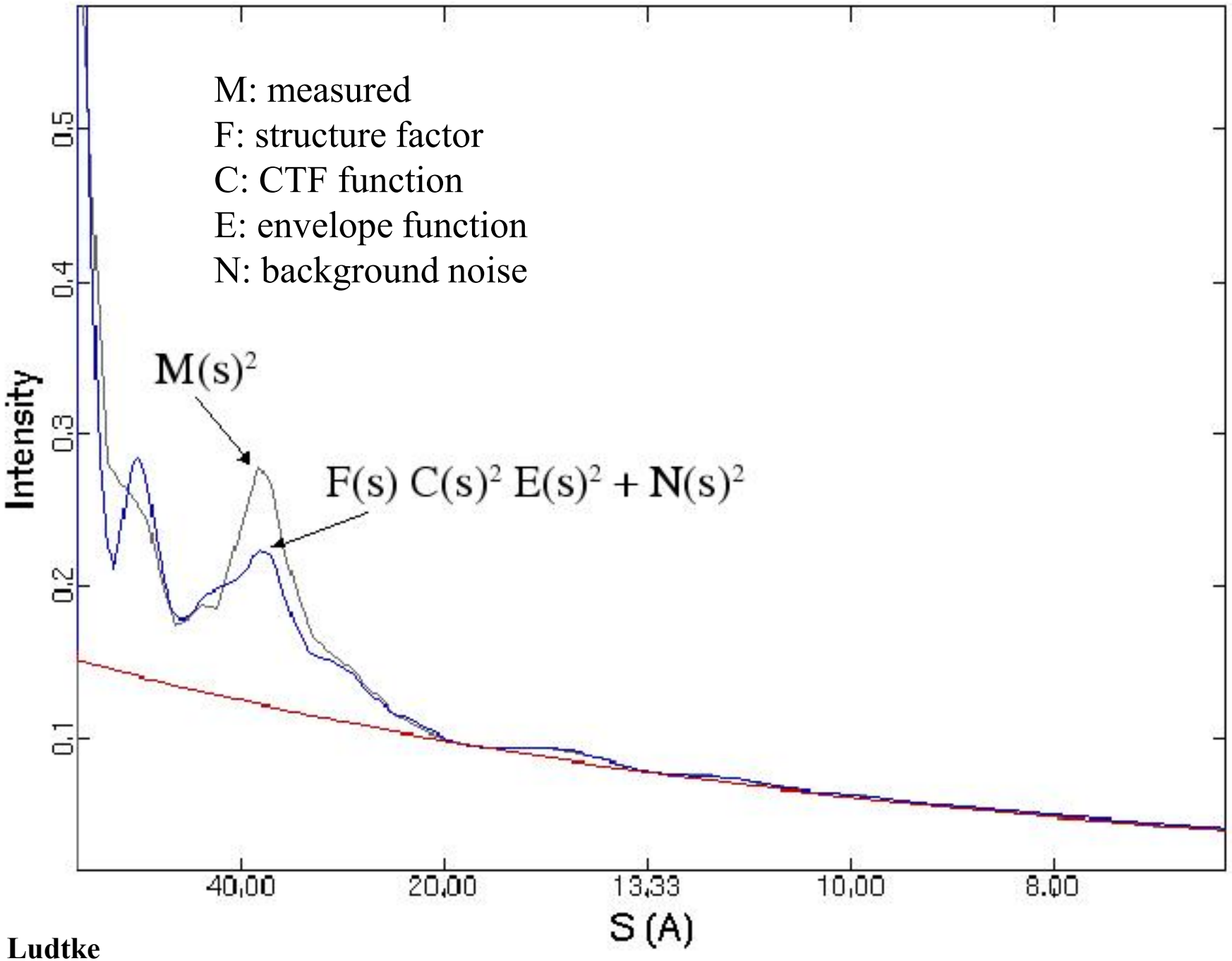
institut de génétique et de biologie moléculaire et cellulaire



From S. Ludtke



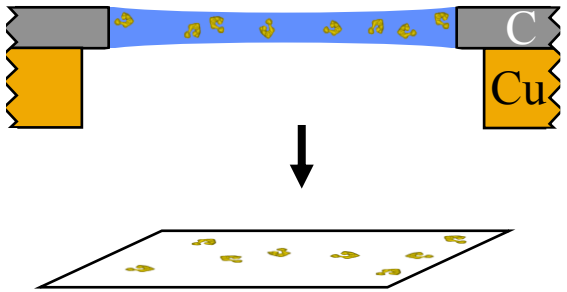
From S. Ludtke

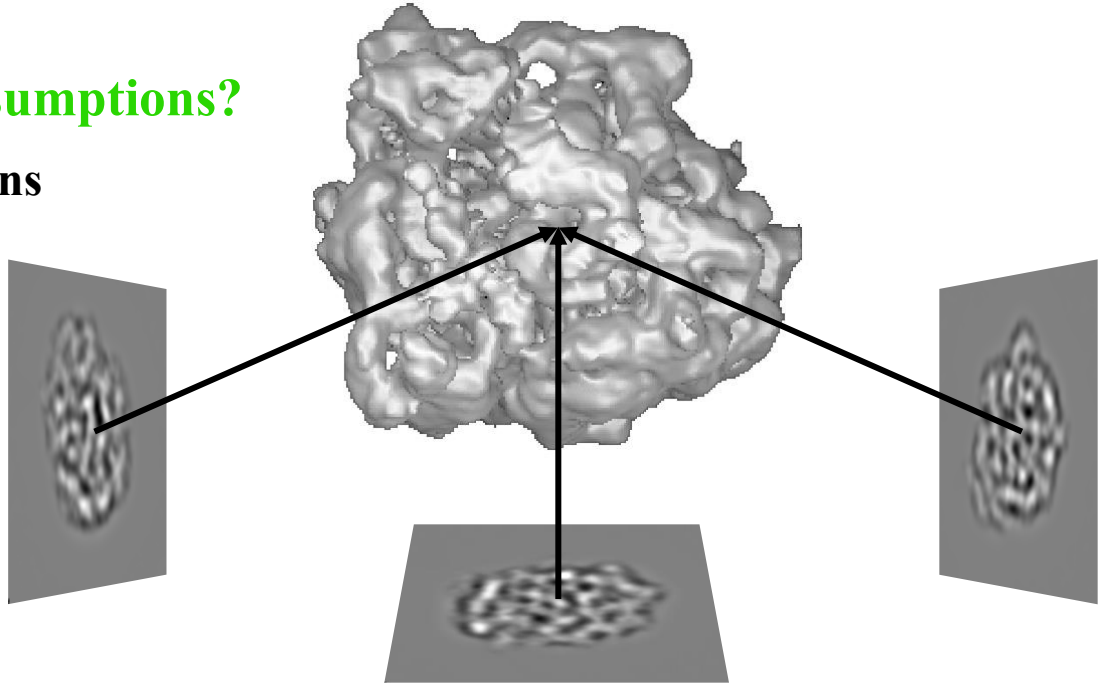
II. Structure determination

- angle assignment
  - angular reconstitution (in early stage of structure determination)
  - projection matching (if structure already well refined): find best correlation between input image and reference images from 3D re-projections)

3D reconstruction of single particles: **assumptions?**

unique particle type in random orientations





Real space *Fourier space*

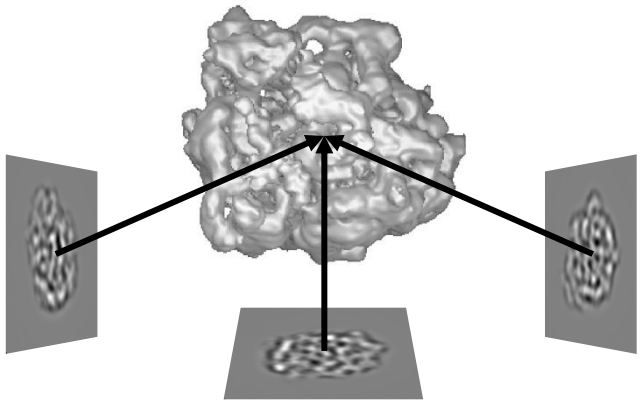
map 3d density distribution 3D FFT 3D Fourier transform

back-project project extract central section insert

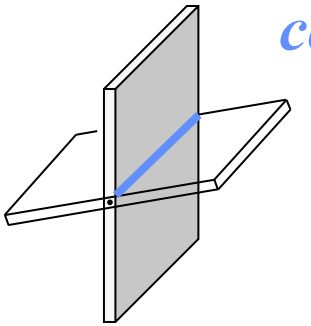
class average 2D projection 2D FFT 2D central section

back-project project to line extract central line insert

sinogram line 1D projection 1D FFT 1D central line



common line



common line projections theorem

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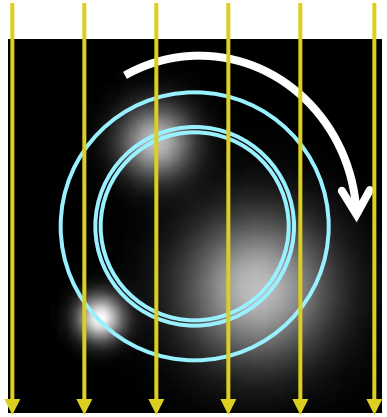
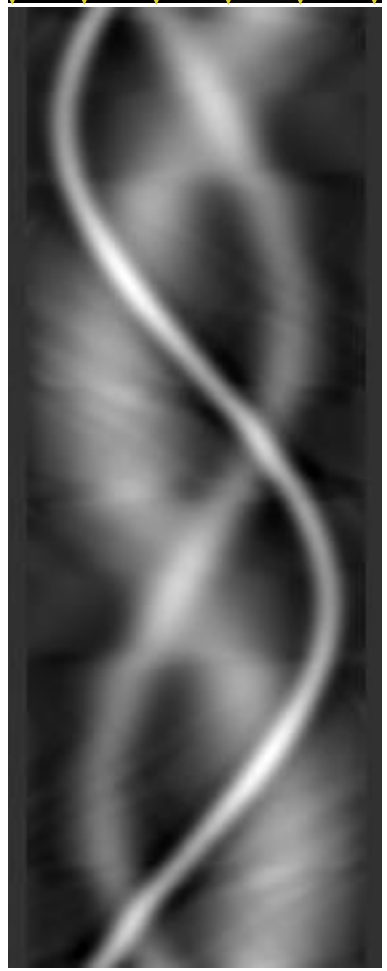
II. Structure determination

- angle assignment
    - angular reconstitution



1

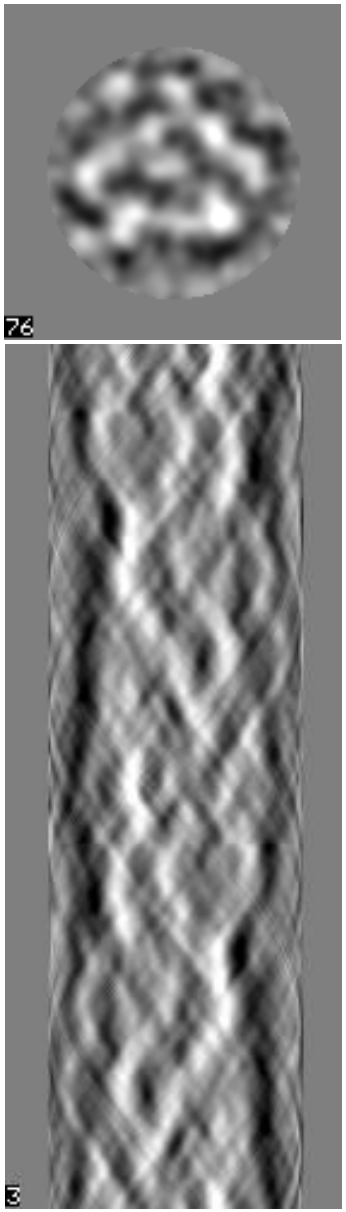


360

amplitude-square-root filtered

sinogram = line-projection of the 2D image (also called Radon transform)

Select 3 clearly different views (here: class average numbers 1,48,76):



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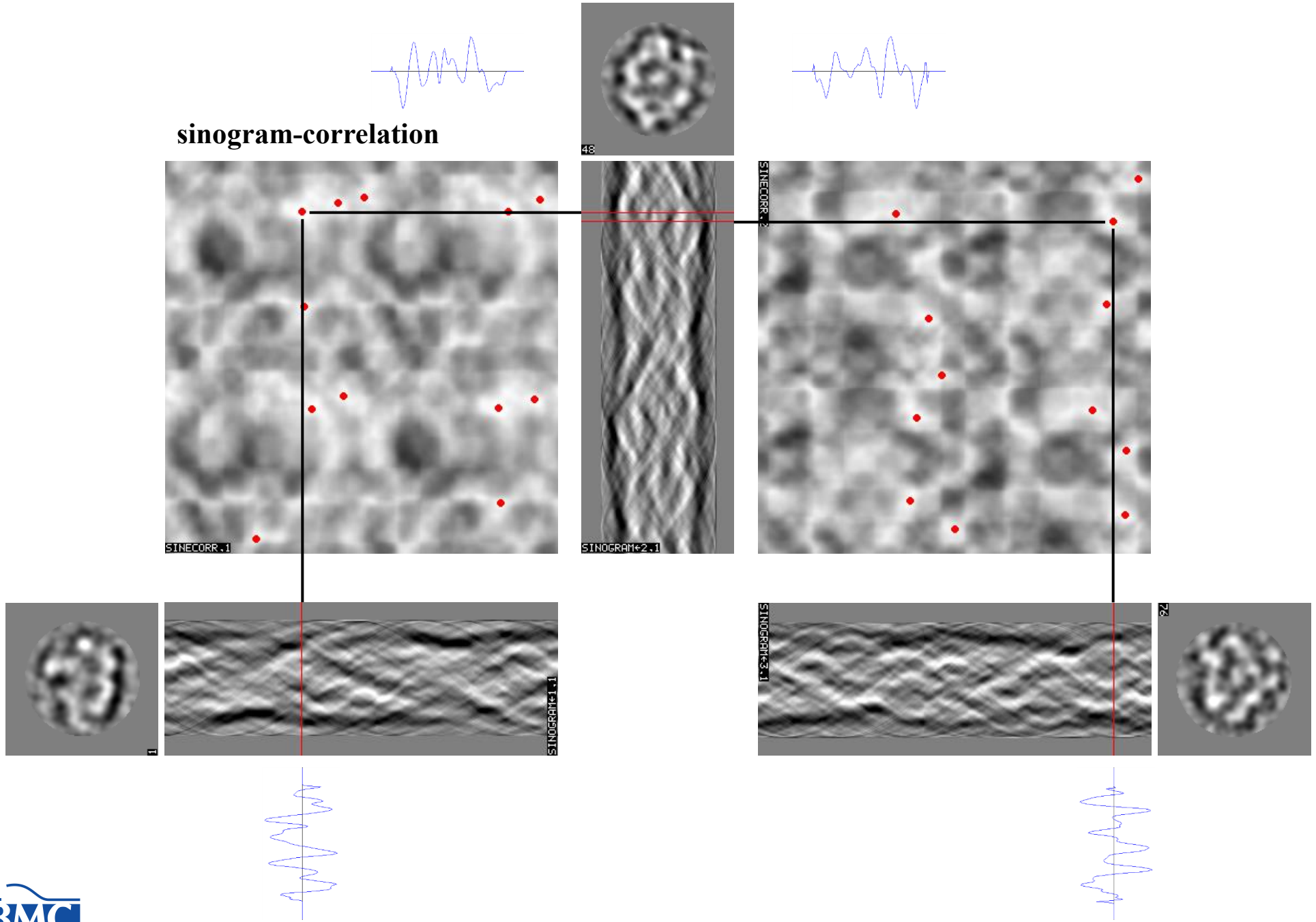
sinogram-correlation



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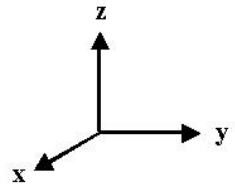
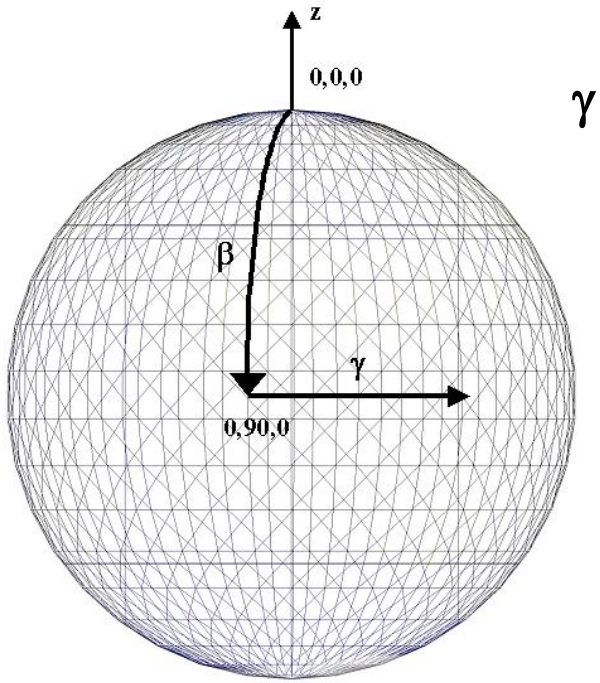
II. Structure determination

- angle assignment
  - angular reconstitution

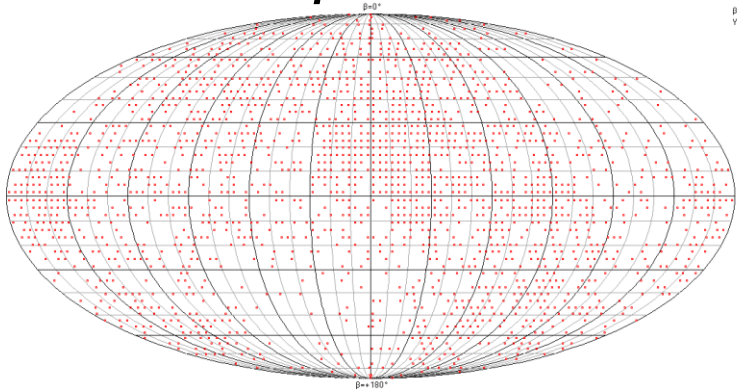




Particle angles plotted on sphere:

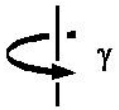
β = 0°

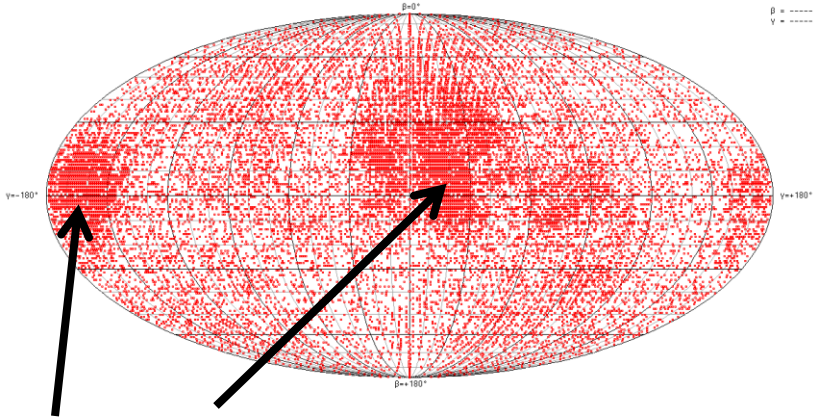
γ = -180°



γ = 180°

β = +180°





Preferential views

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II. Structure determination

- angle assignment
    - angular reconstitution

In case of *ab initio* structure determination by

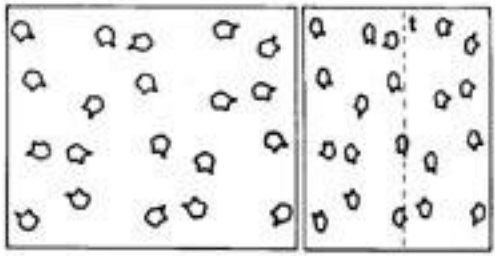
reference-free alignment and angular reconstitution:

Does not allow to determine handedness, requires either:

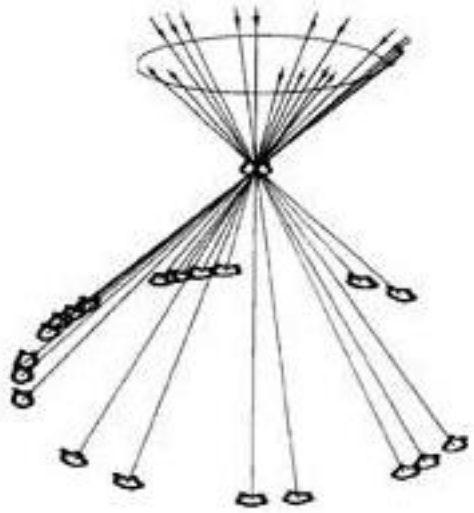
- **random conical tilt (Radermacher *et al.*, J. Microsc. 1987)**
- **tomography**
- **phase residual error using a tilt pair (Rosenthal & Henderson, JMB 2003)**
- **fitting of crystal structures**

II. Structure determination

Random Conical Tilt method (Radermacher 1987)



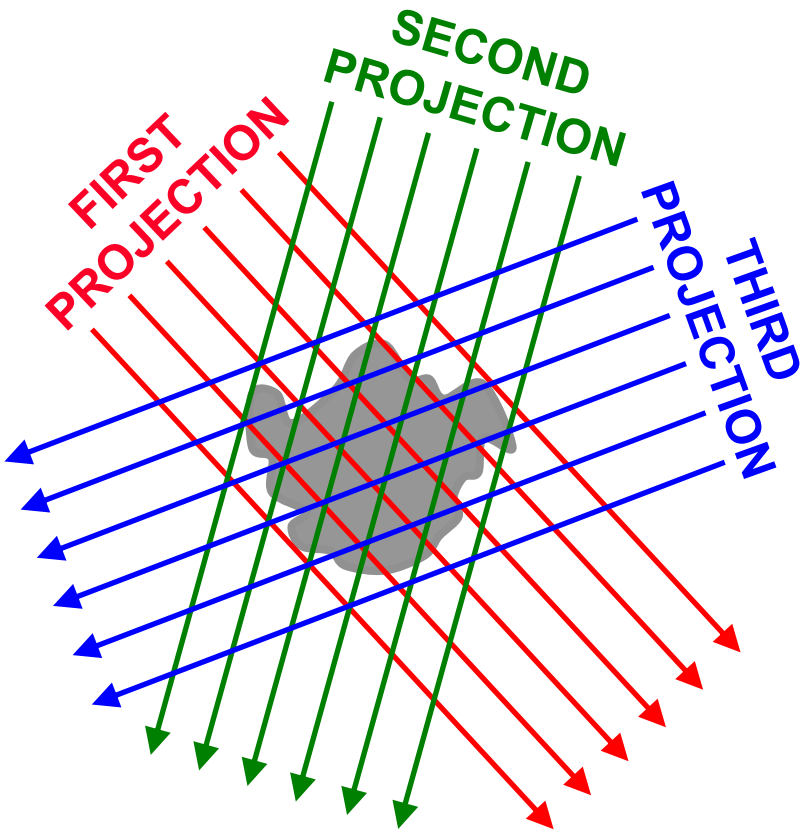
Tilt pair (45deg/0deg); tilt axis

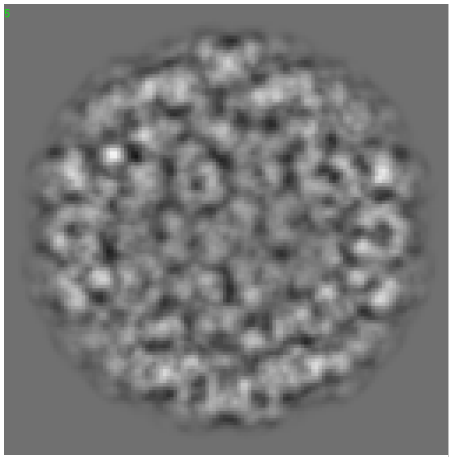


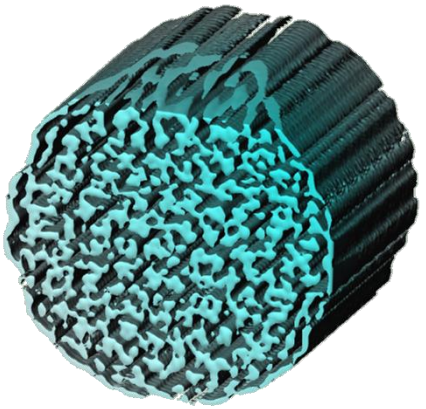
THE “cone””

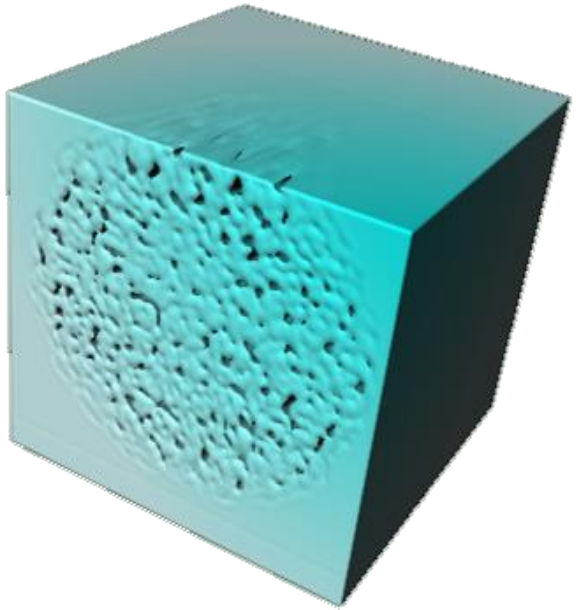
II. Structure determination

- 3D reconstruction









drawn by
I. Orlov

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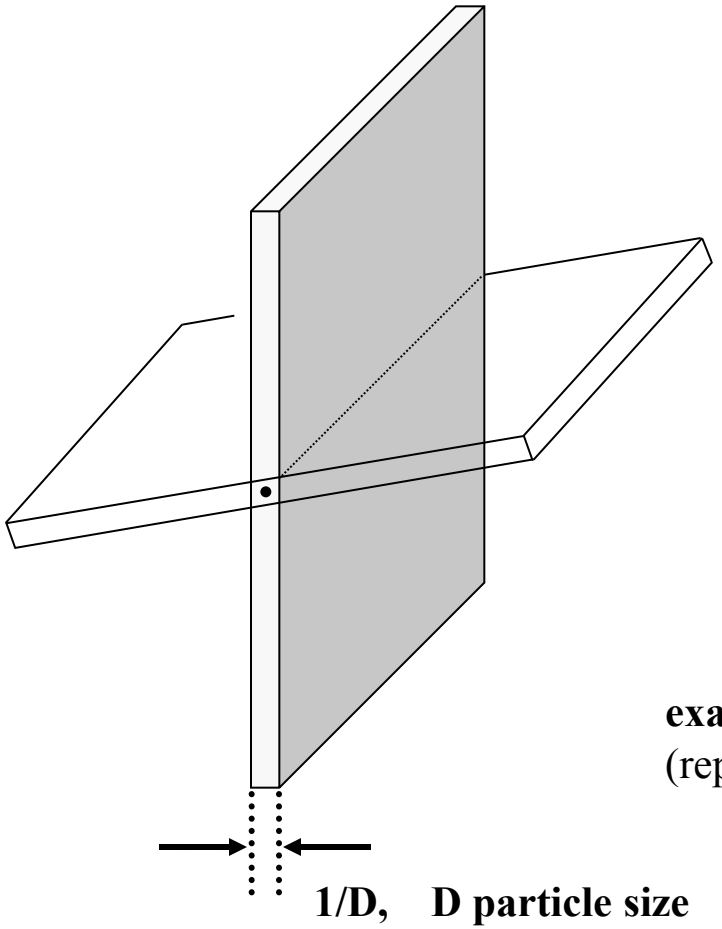
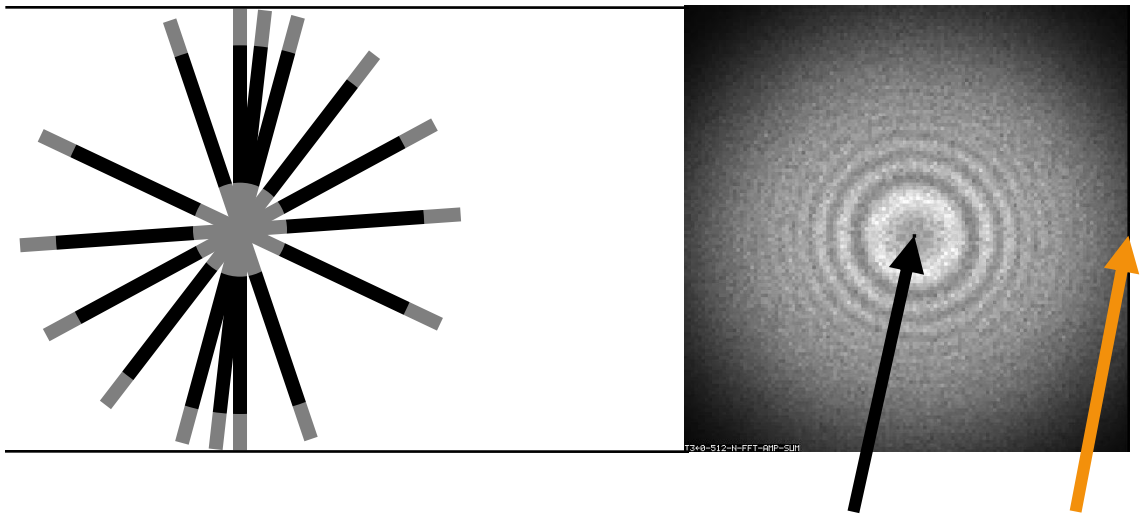
II. Structure determination
- 3D reconstruction



1/D, D particle size

Reminder: powerspectrum:



exact, weighted <u>filtered</u> back-projection
(represented 2D for simplification)

low resolution high resolution
low frequency high frequency

(representations of central sections in Fourier space)

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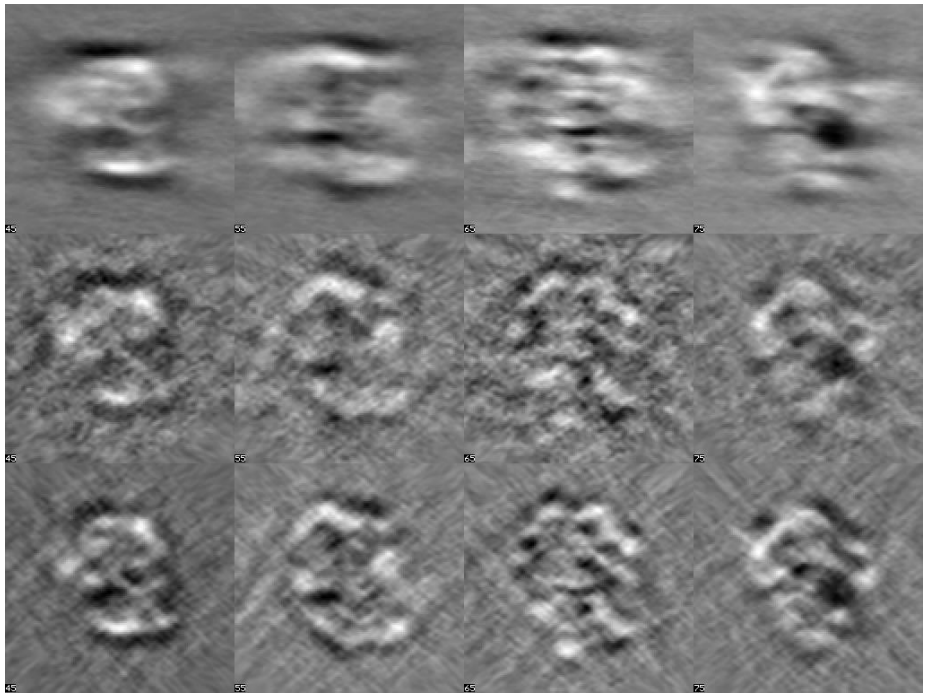
II. Structure determination

- 3D reconstruction

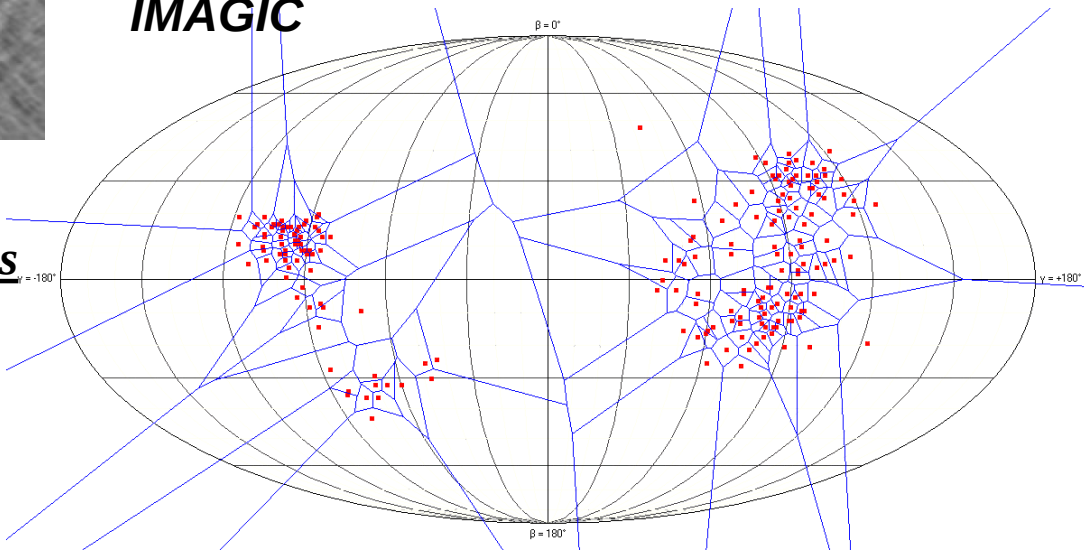


sections of 3D's calculated from 250 class averages with strong preferential views

non-weighted BACK-PROJECTION

Weighted BACK-PROJECTION
~~waited~~

IMAGIC



Orlov *et al.*, in prep. (2014).

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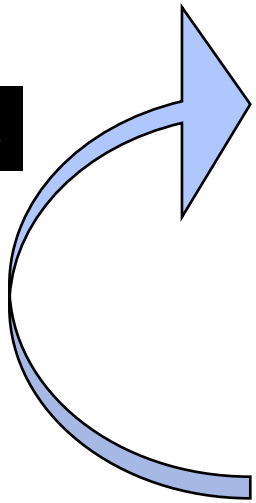
II. Structure determination
- structure refinement

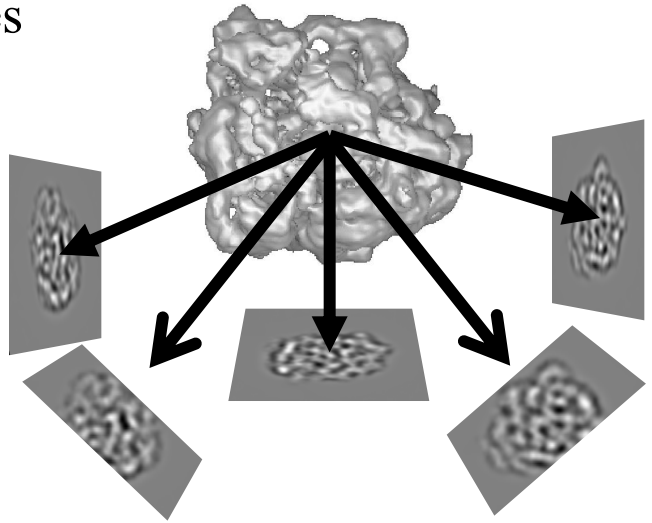
iterations



- centering/alignment
- variance analysis + classification
- angle assignment
- angular reconstitution → 3d-reconstruction
- reprojections = new references

- improve quality of angle assignment

- improve quality of particle alignment



equally distributed forward-projections
(re-projections)

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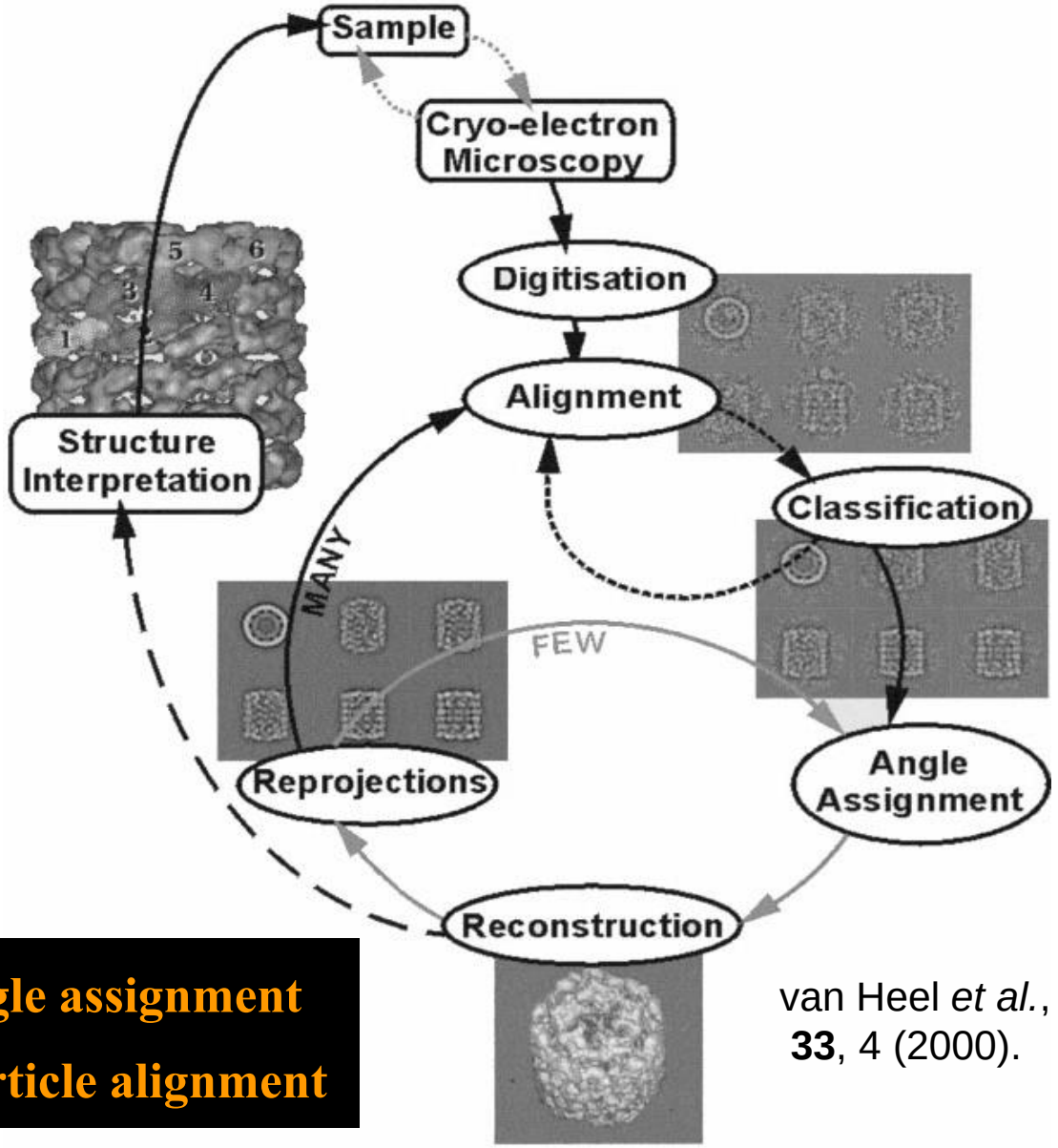
II. Structure determination

- overview



- improve quality of angle assignment

- improve quality of particle alignment

van Heel *et al.*, Quart. Rev. Biophys. **33**, 4 (2000).

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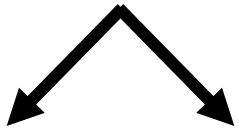
II. Structure determination
- resolution assessment

Particle data set



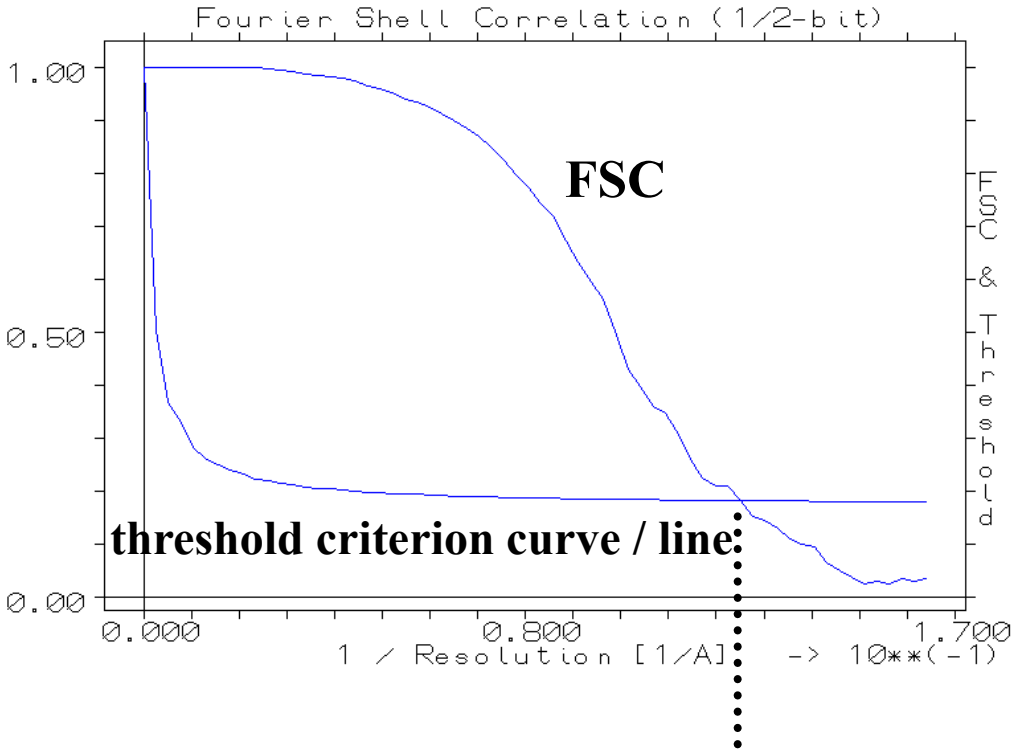
split into 2 halves

3D **3D**

calculate cross-correlation

by shells in Fourier space

Fourier Shell Correlation (FSC)



1 / 1.25 x 1 / 10 Å = 8 Å

Keep in mind: resolution is what you can resolve in the 3D map!

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II. Structure determination

- map interpretation ; fitting of crystal or NMR structures

Fitting procedures:

- manual fitting (e.g. O, A. Jones, Acta Cryst. (1991))

- real space fitting

- reciprocal space fitting

1) global search

2) refinement

e.g. torsion-angle molecular dynamics

- fit complete structures, domains, factors;

Usually backbone is enough.

rigid body or flexible fitting

- use full maps or difference maps

Be careful with local minima and over-fitting!

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Cryo Electron Microscopy

A Titan Krios cryo electron microscope will be installed during 2013. This is the latest generation electron microscope with capacities for high-resolution data collection for both Cryo Electron Tomography (CET) experiments and Single Particle Analysis (SPA) with automated data collection. The electron source is a Field Emission Gun (FEG) that can be operated at 80keV, 100keV, 200keV or 300keV. An automatic loading mechanism allows mounting twelve grids at a time. This microscope is equipped with a CMOS (FEI FALCON 4K*4K) high-sensitivity direct electron detector camera.

The Tecnai F30 Polara cryo electron microscope allows high-resolution SPA and CET automated data collection. Its FEG is usually tuned to 100keV, 200keV or 300keV. The grids are manually mounted 6 at a time. This microscope is equipped with 3 digital cameras: FEI CMOS 4K*4K "FALCON", FEI CCD 4K*4K "EAGLE" and a GATAN CCD 2K*2K "ORIUS".

The Tecnai F20 is equipped with a FEG operated at 100keV or 200keV. Grids are mounted one at a time using a Gatan side-entry cryo-holder. This microscope is equipped with one digital camera (GATAN CCD 2K*2K "US10001"), it is used to collect data for cryo-SPA or room temperature electron tomography using a Fischion side-entry holder.

Cryo-EM grids are flash-frozen in a temperature and humidity controlled environment using a Vitrobot system (FEI).

Although all our microscopes are equipped with digital cameras, we can still record images on films and scan them with a high-resolution Heidelberger Druckmaschinen drum scanner (5 micron pixelsize)

SCIENTIFIC CONTACT

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OTHER PLATFORMS

Sample preparation

- Bacterial expression
- Baculovirus expression
- Yeast expression
- Purification
- Mammalian expression

Biophysical characterisation

- Analytical Ultra Centrifugation
- Calorimetry
- Thermal Shift Assay

Thank you for your attention